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CALORIMETRIC STUDIES OF THE INTERACTION BETWEEN DNA AND POLY-L-LYSINE

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The transition enthalpy ΔH of the helix–random coil transition of the DNA–polylysine complex was measured as a function of the peptide:nucleotide ratio by the help of an adiabatic scanning differential calorimeter. Furthermore the transition enthalpy of a complex with a specific peptide:nucleotide ratio was determined as a function of the cation concentration of the solution. Finally the reaction enthalpy of the interaction of polylysine with native and denatured DNA was measured with the help of a LKB batch calorimeter. From the results of the calorimetric measurements one can conclude that the transition enthalpy of the DNA–polylysine complexes is linearly dependent on the nucleotide: peptide ratio. The extrapolated value for the 1:1 complex is 14.4 kcal per mole base pairs.

1. Introduction

Basic polypeptides especially polylysine share with histones the ability to inhibit in vitro transcription of DNA completely [4]. Therefore polylysine was chosen as a proper histone model at least for histone H_1. It leads to investigations of the physico-chemical properties of complexes between synthetic cationic homopoly peptides and DNA or synthetic polynucleotides [1–3]. One important result of all these measurements is that the formation of such complexes stabilizes the double helix of DNA against thermal denaturation [5,6]. Reported here are the results of direct calorimetric measurements of the enthalpy of the helix–coil transition of DNA–polylysine complexes as a function of peptide to nucleotide ratio and of the ionic strength of the solution. The results of the calorimetric measurements of the enthalpy of the reaction of polylysine with native or denatured DNA under various conditions are also given.

2. Materials and methods

Materials: Calf thymus DNA was lot No. 3 CA, Worthington Biochemical Corporation, N.J. Stock solutions were prepared by gentle stirring of the lyophilized DNA in buffer solution (0.01 M phosphate, pH 7.0) at 4°C for 72 h. Concentrations of DNS solutions were determined gravimetrically by an improved version of the method of Asmus and Baumer [8] before and after each calorimetric measurement. Poly-L-lysine · HBR was lot L 92 from Pilot Chemicals Inc. Watertown, Mass. The average Mw. of polylysine was 70000 daltons. D-L-lysine · HCL was obtained from Fluka AG, Switzerland. Poly-L-lysine·HBr was partially labelled with C 14 (50 μCi per 100 mg polylysine). The concentration of the polypeptide was determined by measuring the rate of radioactive disintegration with a liquid scintillation counter, Tricarb of Packard Instruments, USA. The lysine: phosphate ratios reported are those of the calormetric solutions before the calorimetric measurements. In order to achieve the desired DNA–polylysine ratio, calculated amounts of the two components were mixed in the same buffer. This was done by pressing the polylysine solution through a glass capillary into the stirred DNA solution which yielded a more or less turbid solution. This solution was immediately filled into the calorimeter vessel. The details of the adiabatic calorimeter used in this investigations are given elsewhere [9]. The heat of reaction of DNA with polylysine in dilute aqueous solution was measured with an LKB batch calorimeter (LKB, Bromma, Sweden). DNA was denatured by heating the

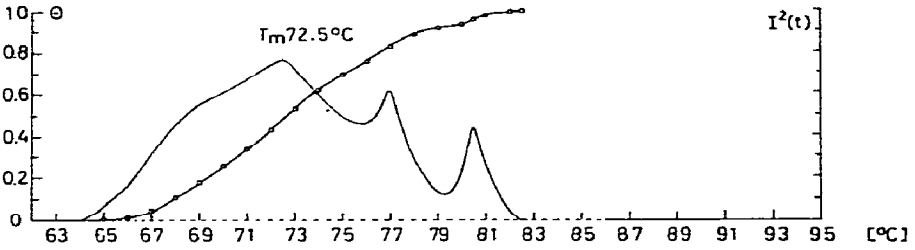

Fig. 1. Heat-induced helix–coil transition curve of calf thymus DNA in 0.01 M NaCl plus 0.01 M sodium phosphate buffer, pH 7.0. Left-hand ordinate: degree of transition θ, computed from the experimental curve (o–o). The DNA concentration in this experiment was 5.0×10^{-3} M(P). Solid line (—): compensating energy, required to maintain adiabatic conditions. Right-hand ordinate: compensating energy, $I^2(t)$.

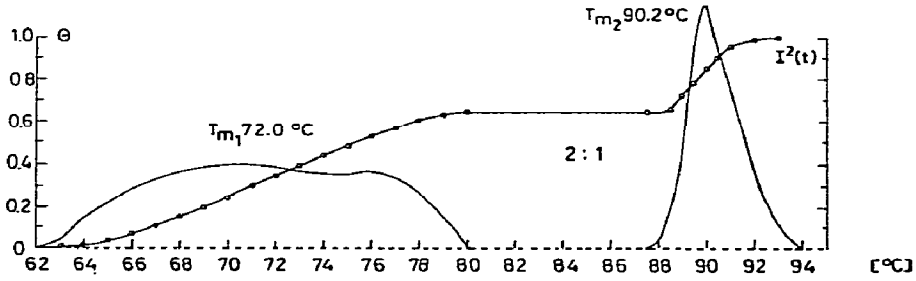

Fig. 2. Heat-induced helix–coil transition curve of calf thymus DNA–poly–L–lysine complex (ratio 2:1) in 0.001 M NaCl plus 0.01 sodium phosphate buffer, pH 7.0. Left-hand ordinate: degree of transition θ, computed from the experimental curve (o–o). The DNA concentration in this experiment was 5.0×10^{-3} M(P). Solid-line (—): compensating energy, required to maintain abiabatic conditions. Right-hand ordinate: compensating energy, $I^2(t)$.

solution to 95 deg. centigrade for 15 min, followed by rapid cooling. No detectible renaturation occurred during the time of the calorimetric measurements. This was confirmed by measuring the UV absorbance as a function of time.

3. Results

When heating native calf thymus DNA at pH 7.0 in 0.01 M phosphate buffer and DNA–polylysine complexes of varying lysine:nucleotide ratios in the same buffer solution, the results shown in figs. 1 and 2 are obtained. The area between the dashed base line and the solid line representing the temperature course of the electrical compensating energy needed to maintain adiabatic conditions in the calorimeter is proportional to the transition enthalpy of the helix random coil transition of the DNA in the solution. The curves with open circles represent the calculated degree of transition θ as a function of temperature for the same temperature interval.

Experimental conditions are given in the legends of the figures. Divergent from the curve of the pure native DNA the curve of the DNA–polylysine complex shows a main peak representing the transition enthapy of the uncomplexed DNA at the same temperature as in fig. 1 and an additional peak at a higher temperature representing the transition enthalpy of the totally complexed DNA. The melting of this complex could also be shown by UV-optical measurements but the concentrations of the polymers were hundred times lower than in the case of the calorimetric measurements.

The dependence of the transition enthalpy on the lysine:nucleotide ratio is shown in fig. 3.

There is a distinct increase of the transition enthalpy

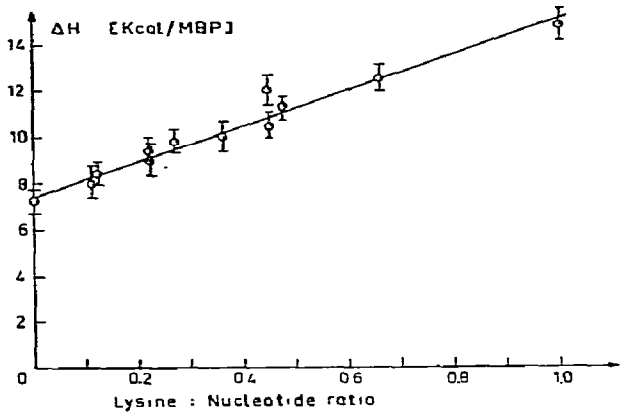

Fig. 3. Plot of the transition enthalpy ΔH versus the lysine: nucleotide ratio. extrapolated to the transition enthalpy of 1:1 complex.

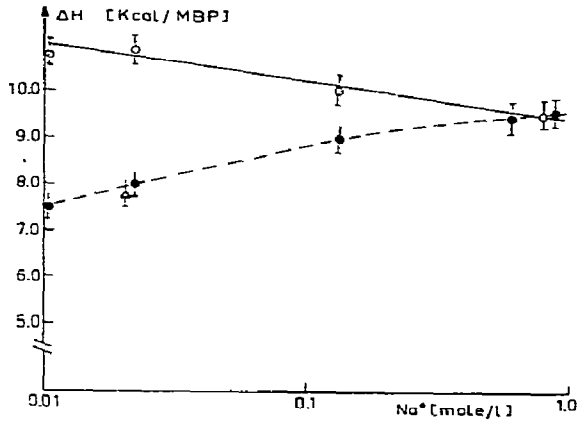

Fig. 4. Plot of the transition enthalpy ΔH versus the logarithm of the sodium ion concentration of the supporting electrolyte. Straight line: DNA–polylysine complex (2:1), dashed line: DNA (data by Gruenwedel [13]). The traingle ▵ shows the experimental value for a DNA–monolysine complex (2:1).

from 7.6 kcal per mole base pairs for the native DNA to about 12.4 kcal per mole base pairs for the phosphorus:lysine ratio of 1:0.6. The dependence of the transition enthalpy of a complex (○) with a phosphorus–lysine ratio of 1:0.44 on the cation concentration (given in a logarithmic scale) is given in fig. 4.

The lower curve represents (●) the transition enthalpy of the corresponding DNA solutions under the same experimental conditions (values taken from the results of Gruenwedel [13]). Both lines tend to the same threshold value. With increasing sodium concentration the stabilizing influence of the polylysine is reduced and finally cancelled. The triangle represents a value obtained for a measurement of a DNA–lysine complex (lysine in the monomeric form), with the same phosphorus–lysine ratio as in the polymer–polymer complexes.

To prove whether the transition enthalpy depends on the interaction enthalpy of polylysine with native or denatured DNA or not the heat of reaction of native or denatured DNA with polylysine solutions were measured at 25°C by the help of a LKB batch calorimeter. The heat of reaction between native DNA and polylysine at 25°C is − 0.55 kcal per mole phosphorus for all lysine:phosphorus ratios greater than one. The heat of reaction between denatured DNA and polylysine at 25°C is + 0.35 kcal per mole phosphorus for the same lysine:phosphorus ratios as given above. There is a difference in the interaction enthalpies of 0.9 kcal per mole phosphorus.

4. Discussion

The interactions of poly-L-lysine with DNA have been studied as a model system [1] of the interaction of a nucleic acid (polyanion) with a basic protein or a basic part of a protein (polycation). When these two polyelectrolytes with opposite charges are mixed in a dilute aqueous solution under certain conditions complexes with a stoichiometric mole ratio are formed. The character of these complexes depends strongly upon the method of preparation. Fast mixing results in a complete coverage of DNA by the added polylysine. The binding is rapid, quantitative and irreversible at low salt concentration and temperature [1]. At a lysine:phosphorus ratio less than unity two species of molecules exist in the solution, native DNA helices and complexes with polylysine where 100 percent binding is assumed.

The other method to prepare the complexes uses a gradient dialysis technique [3]. This way of a stepwise constitution of the complex leads to a uniform distribution of the polylysine among all polynucleotide chains. This procedure usually starts with high salt (e.g., 2 M NaCl) and in the presence of urea [11] and changes gradually to low salt for fixation. In our experiments the first method of preparation was prefered.

Thermal denaturation studies of soluble complexes of poly-L-lysine and nucleic acids with spectrophotometrical methods yield one corresponding result: a reduced hyperchromic effect is found at a normal T_m value of the native DNA besides a second hyperchromic effect at a higher temperature due to the melting of the complex of DNA and polylysine. A higher T_m corresponds to a higher transition enthalpy [12]. The percentage of the hyperchromicity remaining at T_m of native DNA is related linearly to the amount of polylysine added. If we assume 100 percent binding for DNA and polylysine we can calculate the fraction of DNA totally bound in the complex and the fraction of DNA entirely free. If we calculate the transition enthalpy for the "low temperature peak" in fig. 2 under the assumption that at this temperature only native DNA melts then we always get the same value namely 7.6 kcal per mole base pairs. If we assume that the other fraction of the DNA melts as a 1:1 complex with polylysine we obtain the transition enthalpy for the complex namely 14.4 kcal per mole base pairs of DNA.

The total transition enthalpy referred to a base pair of the DNA increases with increasing lysine:nucleotide ratio. When the transition enthalpy per mole base pairs is plotted against the lysine:nucleotide ratio, an almost straight line is obtained as shown in fig. 3. This fact is a strong support for the assumption of a stoichiometric mole ratio of the polyelectrolytes within the complex. The extrapolated value for a lysine: nucleotide ratio is 14.4 kcal per mole of base pairs of DNA.

The stabilizing influence of the polycation on the helix structure of the polyanion is normally assumed to be predominantly electrostatic. However, this assumption seems to be rather inadequate in explaining the special feature shown in fig. 4 (lower curve), i.e., that lysine·HBr monomer of the same lysine: phosphorus ratio has no detectable influence on the transition enthalpy. The addition of neutral salt as sodium chloride to the calorimetric solution decreases the transition enthalpy of a given complex (fig. 4, upper curve) as a function of the log of the sodium ion concentration. H for the complex (lysine:phosphorus ratio of 0.44) amounts to 10.8 kcal/MBP at a sodium ion concentration of 10 mM. When the cation concentration is raised to 0.78 M Na^+ the transition enthalpy decreases to 9.5 kcal per mole base pair. This value corresponds to the result of Gruenwedel [13] obtained for the transition enthalpy of calf thymus DNA in a saline aqueous solution of the same sodium concentration. The lower curve in fig. 4 shows Gruenwedels results for pure DNA solutions [13]. It is clearly shown from this figure that the influence of polylysine on the structural stability of the DNA helix is cancelled at a cation concentration of 0.6 M Na^+. This result is in good agreement with the finding of Matsuo and Tsuboi [14] that the melting temperature T_m of the poly (I + C) helix which serves as DNA model and of the polylysine–poly (I + C) complex depends upon the salt concentration. The dissociation of nucleohistones into histones and DNA starts at this salt concentration [15]. In this connection some recent results of Ross [10] and Shapiro should be mentioned. These authors presented the results of calorimetric studies of the interaction between native DNA and polyvalent cations, e.g., polylysine. The lysine concentration was higher than one charge equivalent cation per DNA and the added salt concentration was 0.6 M NaCl in the case of polylysine. The heat of interaction was –300 cal/mol P at 25°C. This is a bit different to the result of –550 cal/mol P presented in this paper obtained for the interaction of polylysine with native calf thymus DNA.

Since two different species of DNA namely salmon sperm DNA [10] and calf thymus DNA in this case were applied in the experiments and the salt concentrations differed a lot the differences of the results may be due to these different conditions. Shih and Bonner [4] have shown that DNA complexed with polylysine or other basic polypeptides is quantitatively blocked from acting as template in support of RNA synthesis. Elongation of the RNA chain during the transcription requires some local unwinding melting of the DNA double helical structure. As could be shown in this investigation, the interaction of polylysine and DNA increases the transition enthalpy markedly compared with uncomplexed DNA under the same set of experimental conditions. It is therefore reasonable to assume that polylysine hinders the local unwinding of the DNA. In case of an increasing ionic strength of the solvent this influence is diminished and the usual transcription mechanism may well be running again and the DNA will be available as a template for RNA synthesis. This is a possible mechanism for the role of basis proteins in regulating the action of genes and chromosomes, as postulated by Allfrey and Mirsky [16].

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